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Scopolamine and KCl Injections into the Caudate Nucleus. Overtraining-Induced Protection Against Deficits of Learning[1]

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PRADO-ALCALA, R. A., P. KAUFMANN AND R. MOSCONA. *Scopolamine and KCl injections into the caudate nucleus. Overtraining-induced protection against deficits of learning.* PHARMAC. BIOCHEM. BEHAV. 12(2) 249–253, 1980.—To test the hypothesis that extended training of an instrumental task prevents the performance impairments seen after cholinergic and generalized blockade of caudate-putamen complex (NC) activity in animals with a relatively low degree of training, groups of rats were trained to press a lever under a continuous reinforcement schedule for 5, 15 or 25 sessions. The effects of microinjections of scopolamine and potassium chloride into the CN were then assessed. In agreement with early studies in cats, a significant deficit in performance was produced in the animals with a low or medium degree of training, while no changes in learned behavior were seen in the overtrained rats. These results show that: (a) normal neural activity of the CN is essential for performance of instrumental behavior during acquisition and early maintenance stages but not after overtraining, and (b) that after extended training the encoding necessary for performance may be transferred to another neural system outside the CN.

Caudate nucleus Learning Cholinergic blockade Scopolamine Potassium chloride Overtraining

AFTER the work of Neill and Grossman [7] showing that the cholinergic blockade of the caudate-putamen complex (CN) induces significant deficits of an active avoidance task, other investigators have also found equivalent impairments of different instrumental tasks upon the application of anticholinergic drugs into the caudate [2, 3, 8, 12], thus supporting the hypothesis that cholinergic activity of this structure is critically involved in both the acquisition and maintenance of instrumental learning.

We have recently shown that cholinergic blockade of the caudate nucleus in cats induces a marked impairment in the performance of a relatively simple lever-pressing task, trained for a brief period under a continuous reinforcement (CRF) schedule, while the same treatment is ineffective when applied after a period of overtraining [10]. The same overtraining-induced protection against behavioral deficits was observed in rats, working under the effects of microinjections of scopolamine into the CN, on a more complex task [8]. A functional change within the caudate nucleus, which is dependent on the amount of training, was proposed to explain the results of an experiment in which the effects of potassium chloride (KCl) injections into it were assessed in cats with low, medium and high degrees of training. A complete abolishment of learned behavior was produced in the least trained animals while near perfect responding was seen in the highly trained group [9].

These results have led us to postulate two complementary hypotheses: (a) that after extended training cholinergic activity of the caudate nucleus is not essential for instrumental performance, and (b) that after overtraining the encoding necessary for instrumental performance is transferred to other neurochemical systems within the caudate or to other regions of the nervous system.

The aim of this study was two-fold: (1) to further test these hypotheses, and (2) to extend our previous studies in cats to another species.

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METHOD

Animals

Eighty-four experimentally naive male rats of the Wistar strain, weighing between 200 and 300 g were used. They were individually lodged and had free access to solid food (Purina Laboratory Chow) throughout the experiment. Under Nembutal anesthesia (45 mg/kg), some animals were bilaterally implanted with permanent double-walled cannulae (21 and 27 hypodermic needle tubing). Postmortem histological analysis (Nissel stain) revealed that the tips of the cannulae were located either in the dorsal half of the anterior region of the CN, between A-P 7.0 and 7.9 (n=28) or in the parietal cortex, within the A-P limits mentioned for the caudate animals (n=6). Stereotaxic coordinates were obtained from the König and Klippel atlas [6].

Apparatus

Training was conducted in a conventional Skinner box (Lafayette Instruments Co.) provided with one lever, the depression of which yielded 0.05 ml of tap water. Recordings of pressing rate, water delivery and control of the duration of each session were accomplished by use of electromechanical programming equipment. The Skinner box was located inside a sound-attenuating room kept under constant dim illumination.

Procedure

All animals were deprived of water for about 23 hr prior to each session. On the first session rats were shaped by the experimenter to press the lever in order to be rewarded with water; the session ended when rats pressed the lever 10 to 15 times. For the remainder of the experiment each daily session lasted 12 min, and there were 5 consecutive sessions per week (Monday through Friday). Thus, different groups of animals were trained for 5, 15 or 25 sessions following a continuous reinforcement schedule (CRF) by which each lever press was followed by the delivery of water.

Groups

There were three groups of unimplanted rats that were trained for 5 (group UI-5, n=28), 15 (UI-15, n=16) or 25 (UI-25, n=6) sessions; three groups of animals implanted in the caudate-putamen that were also trained for 5 (CN-5-SK, n=8), 15 (CN-15-SK, n=7) or 25 (CN-25-SK, n=6) days and that were microinjected with scopolamine and potassium chloride. Two additional groups were trained for 5 sessions, one implanted in the CN and treated with saline solution and KCl (CN-5-NaCl, n=7) and the other implanted in the parietal cortex and microinjected with scopolamine and KCl (CTx-5-SK, n=6). Rats were randomly assigned to each group.

Two to five hours after the last training session the CN and the cortical groups were submitted to cannulae implantation as described above, and starting on the third post-implantation day all groups were tested on the same CRF schedule for 8 additional sessions. Unimplanted animals were also tested for the same number of days.

Treatments

Microinjections were performed outside the experimental room, were always bilateral, and the solutions delivered at a rate of 1 μl/20 sec. After injecting, the delivery cannulae were kept inside the implanted cannulae for an additional minute, allowing for better diffusion through neural tissue. Depending on their group's membership, 6 min before the 4th testing session implanted animals were microinjected with 20 μg of scopolamine dissolved in 3 μl of saline solution (NaCl) or with the same volume of NaCl through each cannula. KCl, in a volume of 1 μl, was microinjected into each structure in all implanted animals, 6 min before the 7th session. On Days 1, 2, 3, 5, 6 and 8 no treatments were given and the same testing (CRF) procedure was conducted as usual.

The scopolamine bromide (Merck) solution had 252.0 mOsm and a pH of 5.9; the NaCl solution had 217.3 mOsm and a pH of 5.8. Microinjections on the 7th testing day were made with a 3 molar KCl (J. T. Baker) solution.

Statistics

Comparisons of pressing rates among the groups were made using analysis of variance (F test), and between pairs of groups using *t* tests for independent samples. The study of each group's performance, across sessions, was accomplished with *t* tests for related samples. Probability values are one-tailed.

RESULTS

Performance on Training Sessions

As expected, none of the groups trained for 5 sessions (CN-5-SK, CN-5-NaCl, Ctx-5-SK and UI-5) differed from each other in their performance on any of the sessions prior to cannulae implantation; the same held true for both groups trained for 15 days (CN-15-SK and UI-15) as well as for those trained for 25 sessions (CN-25-SK and UI-25).

Figure 1 shows acquisition curves for the combined data of the 5 sessions groups, the 15 session groups and the 25 session groups, respectively. It is evident that, as expected, all groups of rats behaved very similarly during the first five days of training ($F=0.363$ [2,80], N.S., on the 5th session). As the experimental sessions progressed, the 15 session groups and the 25 session groups increased their performance levels in a parallel manner up to the 15th session. Afterwards, the remaining groups did not show any significant improvement in lever pressing. On comparing the performance during the last day of training between all the groups a significant difference appeared ($F=20.060$ [2,80], $p<0.0001$); subsequent *t* tests showed that the 5 session groups had a poorer performance than the 15 ($p<0.005$) and 25 ($p<0.005$) session groups, while the latter two groups did not differ from each other.

Effects of Cannulae Implantation

Analysis of pressing rates on the third testing (pre-treatment) session showed that there were highly significant differences among the groups ($F=6.017$ [7,76], $p<0.00006$). Subsequent comparisons between every combination of

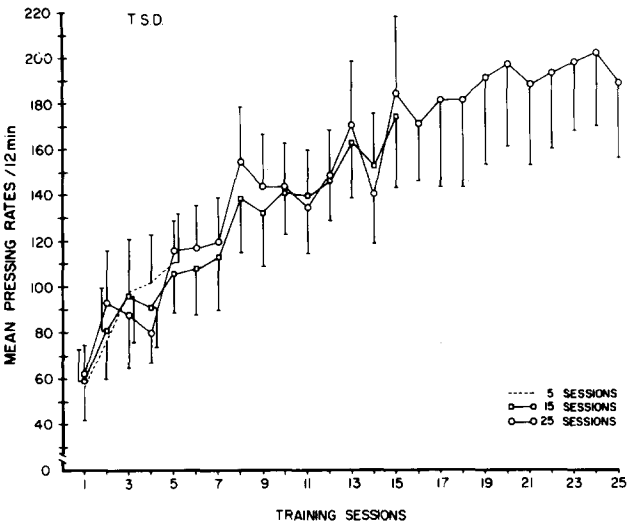

FIG. 1. Mean pressing rates, with their respective standard deviations (SD), measured before cannulae implantation in all groups of rats trained for 5 (----), 15 (*——*) or 25 (○——○) sessions. See text for details.

pairs of groups showed that each of the least trained groups (5 sessions) differed from each of the unimplanted groups trained for 15 and 25 sessions (Fig. 2).

More interesting, however, is the fact that the caudate-putamen (CN-5-SK and CN-5-NaCl) groups trained during 5 sessions had lower pressing rates than their corresponding control (UI-5) group ($p<0.025$ for each comparison), while the group implanted in the cerebral cortex did not show such behavioral decrement. Similarly, the performance of group CN-15-SK was lower than its control (UI-15) group also trained for 15 days ($p<0.005$). In contrast, learned behavior of the group of animals trained for 25 sessions and then implanted in the CN was not reliably different from the unimplanted rats with the same amount of training.

Effects of Treatments

As shown above, there were significant differences in pressing rates among the groups before treatments were begun. This outcome prevented us from further comparisons among groups, and led us to examine only the effects of treatments within each group across testing sessions. For the sake of clarity in the presentation of our results, raw data was transformed to percentage of pressing rates relative to the pre-treatment session described above.

Performance of the groups of rats trained for 5 sessions is depicted in Fig. 3. Stable performance is evident in the unimplanted (UI-5) animals. In group CN-5-NaCl no reliable changes in conditioned responding was observed after microinjecting NaCl. However, after KCl application a marked deficit in performance was induced ($p<0.01$). Similar behavioral deficits were produced in group CN-5-SK by application of scopolamine ($p<0.025$) and KCl ($p<0.001$) in the first and second microinjections sessions, respectively. On the

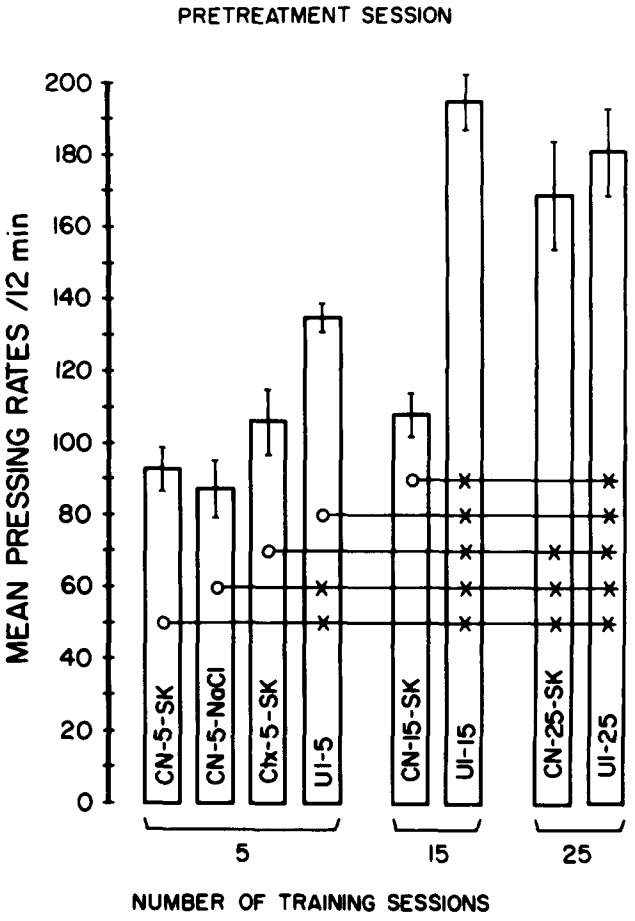

FIG. 2. Mean pressing rates, with their respective standard error, on pre-treatment session of groups trained for 5 sessions (CN-5-SK, implanted in the caudate-putamen complex and treated with scopolamine and KCl; CN-5-NaCl, implanted in the caudate-putamen and treated with NaCl and KCl; Ctx-5-SK, implanted in the parietal cortex and treated with scopolamine and KCl; UI-5, unimplanted controls), 15 sessions (CN-15-SK, implanted in the caudate-putamen and treated with scopolamine and KC1; UI-15, unimplanted controls) or 25 sessions (CN-25-SK, implanted in the caudate-putamen and treated with scopolamine and KC1; UI-25, unimplanted controls). Significant differences ($p<0.05$) in performance between a particular group (0) and another group or groups are represented by an asterisk (*).

other hand, scopolamine microinjections were ineffective in producing alterations in pressing rates when applied into the parietal cortex, although a small but reliable decrement in performance was seen upon injecting KCl in this region ($p<0.025$).

Figure 4 shows that the unimplanted rats trained for 15 (UI-15) and 25 (UI-25) sessions also displayed a stable performance throughout the testing days. A very similar pattern of responding to that of group CN-5-SK can be seen in group CN-15-SK after scopolamine and KCl microinjections, i.e.,

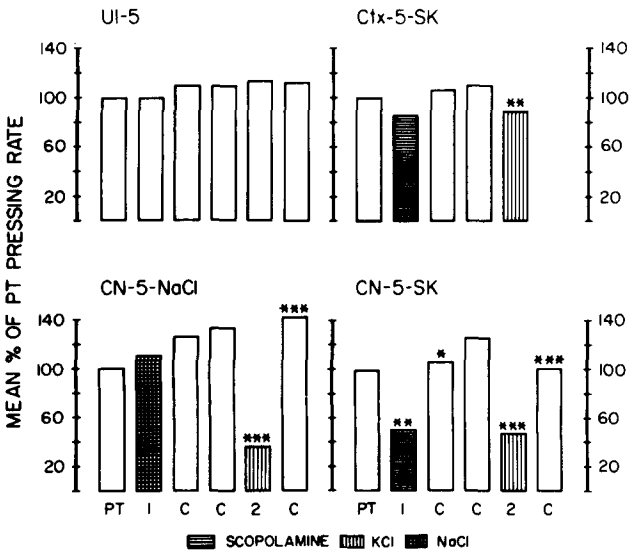

FIG. 3. Mean percent of pretreatment pressing rates for the groups trained for five sessions. PT, pretreatment session; 1 and 2, first and second injection sessions, respectively; C, control sessions. Asterisks represent significant differences between a particular session and the previous session for each group: *, <0.05; **, <0.025; ***, <0.01. Abbreviations for each group are the same as in Fig. 1.

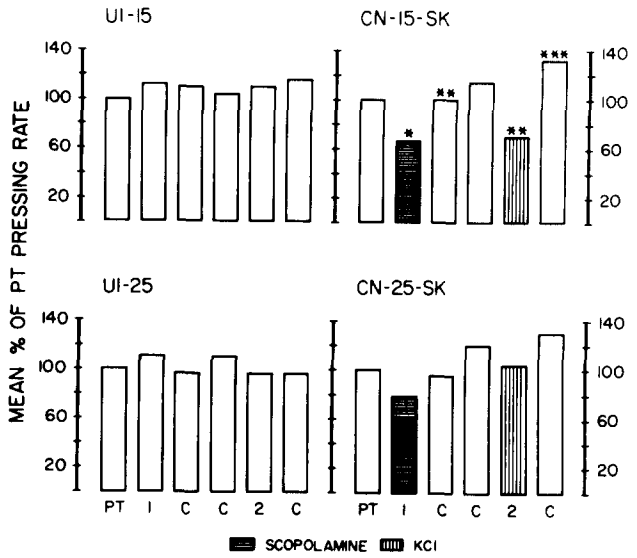

FIG. 4. Mean percent of pre-treatment pressing rates (PT) for the 15 and 25 session groups. Abbreviations represent the same as in Figs. 1 and 2.

after injecting each of the solutions a significant impairment in performance was produced ($p<0.025$ and $p<0.005$, respectively). In sharp contrast, lever pressing was not significantly altered when the effects of these treatments, also applied to the CN, were assessed in rats trained for 25 sessions (group CN-25-SK).

DISCUSSION

Pre-implantation data show that the 5 session groups were still going through the acquisition phase of the learning process, since their performance on their last (5th) day of training differed significantly from that of the last day of training of both the 15 and the 25 session groups. On the other hand, the latter two groups did not differ in their learned behavior from each other on any of their training days; thus, it can be stated that by the 15th session they had reached a stable performance (maintenance) level, as seen in Fig. 1.

The differential rates of responding found in the unimplanted groups during the third (pre-treatment) session was expected since, as stated above, the 5 session group was still going through the acquistion phase of the learning process while the 15 and 25 session groups had reached the maintenance state (as evidenced by the equally assimptotic performance of the latter groups, Fig. 1). The decreased rates of responding seen in the groups of animals trained for 5 and 15 days that were implanted in the CN, and the lack of interference with performance in group CN-25-SK could also be predicted on the basis of a recent experiment in which generalized disfunction of the caudate of cats also induced impairments on CRF performance, which were negatively correlated with the amount of training [9]. In the present case, the relatively small lesions produced by the penetration of the cannulae into CN tissue sufficed to show this effect, which was not due to the surgical procedures per se, since no significant deficits in responding were seen in the cortical group trained for 5 sessions nor, as stated above, in the CN group trained for 25 days. To put it another way, small lesions of the CN induce an impairment in instrumental performance in animals that are studied during the acquisition and early stages of the maintenance of learning, while no effects on learned behavior are produced in animals submitted to overtraining.

As to the cholinergic blockade of the anterior region of the caudate-putamen, the same training-dependent differential effects on lever pressing behavior seen earlier in cats [10] were reproduced in the present experiment. Only rats with a low degree of training and those going through the early maintenance stages of conditioning were significantly impaired. We can exclude the possibility that these effects were due to the handling of the rats during microinjection procedures or to the delivery of a volume into neural tissue since, on the one hand, no interference with responding was seen in the group of animals submitted to the same manipulations and injected with the same volume of saline into the CN (group CN-5-NaCl), and on the other, no significant behavioral deficits were induced by scopolamine applications into the CN of the rats trained for 25 sessions.

The notion that the effects of scopolamine were due to blockade of the dorsal aspect of the anterior caudate, is supported by experiments in which cholinergic blockade of that region was also shown to produce marked deficits in CRF and maze performance in cats [12], as well as in active [7]

and passive [2, 3, 5] avoidance, and in a more complex spatial alternation task [8] in rats. Further support for the specificity of the effects under discussion is provided by the reports that anticholinergic drugs fail to disrupt instrumental conditioning when applied to the posterior [2,8] or to the ventral aspects [7] of the caudate-putamen complex. There is also evidence demonstrating that anticholinergic drugs are ineffective in disrupting learned performance when injected into the cerebral cortex ([8] and in this report), cerebral ventricles [12], dorsal hippocampus [5] or basal amygdaloid nuclei [12].

Also in agreement with results obtained after KCl microinjections into the caudate of cats similarly submitted to low, medium and high degrees of CRF training [2], KCl microinjections into the CN of rats induced a marked decrement in performance only in the low (5 sessions) and medium (15 sessions) trained rats. The fact that KCl, but not scopolamine, applications to the parietal cortex of the rats belonging to group Ctx-5-NaCl also interfered with the conditioned response can be explained by the different effects these agents exert on neural functions. Scopolamine blocks cholinergic transmission while KCl induces a generalized interference with neural activity, as evidenced by slow potential changes, decreased EEG and an initial period of high frequency spike discharges followed by long periods of a markedly decreased frequency of action potentials [1, 4, 13, 14]. Although KCl was applied to a restricted cortical area, there is a spreading of these effects, in the rat, not only to the rest of the cortical mantle, but also to the CN [1]. Similarly,KCl injected into the CN induces the same phenomena which also propagate to the cortex [1].

The small, but reliable, impairment of learned performance seen after KCl injections into the cortex of our rats can be interpreted either as being due to the induced cortical disfunction, to interference with caudate activity, or both. We favor the second proposition since direct microinjections of KCl into the CN (groups CN-5-SK and CN-15-SK) produced by far, a greater impairment in lever pressing than that seen in the cortical group. In addition, KCl applications to the parietal cortex of cats with a low degree of CRF training failed to produce decrements in performance; when applied to the caudate, abolishment of the response was observed [11]. In the cat there is no spreading of the direct effects of KCl in either direction between the cortex and the caudate nucleus [1,13].

The decrements in performance seen upon injecting scopolamine and KCl to the 5 and 15 session groups cannot be accounted for by inteference with motivational, perceptual, motor or other processes which are essential for performance, but to an impairment of memory mechanisms, because the same treatments given to group CN-25-SK did not alter the execution of the learned response nor the gross and fine motor adjustments needed for the consumption of the reinforcers. Along this line, we have recently found that rats under the effects of scopolamine (30 μg in 3 μl/CN) drink the same amounts of water as untreated rats [8].

Congruent with previous reports [8,10] our present data suggest that cholinergic activity of the anterior region of the caudate nucleus is essential for the performance of instrumental behaviors during the acquisition and early stages of maintenance of conditioning, and that after overtraining such activity becomes less involved in performance. The fact that generalized interference with neural activity of the CN, induced by KCl, produced parallel behavioral effects to those seen after scopolamine, also supports the hypothesis that after extended training the encoding necessary for the performance of instrumental learning is transferred to other structure or structures outside the caudate nucleus [9].

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